Chapter 19
The Citric Acid Cycle

SUMMARY

Section 19.1

- The citric acid cycle is amphibolic. It plays a role in both catabolism and anabolism. It is *the* central metabolic pathway.

Section 19.2

- The citric acid cycle takes place in the mitochondrial matrix, with exception that one enzyme is located in the inner mitochondrial membrane. The closely related process of oxidative phosphorylation takes place in the inner mitochondrial membrane.

Section 19.3

- The two-carbon unit needed at the start of the citric acid cycle is obtained by converting pyruvate to acetyl-CoA.
- This conversion requires the three primary enzymes of the pyruvate dehydrogenase complex, as well as the cofactors TPP, FAD, NAD^+, and lipoic acid.
- The overall reaction of pyruvate dehydrogenase is the conversion of pyruvate, NAD^+, and CoA-SH to acetyl-CoA, NADH + H^+, and CO_2.

Section 19.4

- In the citric acid cycle and the pyruvate dehydrogenase reaction, one molecule of pyruvate is oxidized to three molecules of carbon dioxide as a result of oxidative decarboxylations
- .The oxidations are accompanied by reductions. Four NAD^+ are reduced to NADH, and one FAD to $FADH_2$; in addition, one GDP is phosphorylated to GTP.

Section 19.5

- The citric acid cycle is exergonic in terms of overall free-energy changes. In addition, it produces four NADH and one $FADH_2$ for each pyruvate that enters the cycle. Reoxidation of these electron carriers produces 25 ATP.
- Four control points exist for the citric acid cycle. One, the pyruvate dehydrogenase reaction, lies outside the cycle proper. The formation of citrate and the two oxidative decarboxylations are the other control points. ATP and NADH are inhibitors of the cycle, and ADP and NAD^+ are activators.

Section 19.6

- In plants and bacteria, the glyoxylate cycle is a pathway that bypasses the two oxidative decarboxylations of the citric acid cycle. As a result of this pathway, plants can convert acetyl-CoA to carbohydrates, which animals cannot do.

Section 19.7

- All metabolic pathways are related, and all operate simultaneously.
- In catabolic pathways, nutrients, many of which are macromolecules, are broken down to smaller molecules, such as sugars, fatty acids, and amino acids.
- Small molecules are processed further, and the end products of catabolism frequently enter the citric acid cycle, which plays a key role in metabolism.

Section 19.8

- The citric acid cycle plays a central role in anabolic pathways as well as in
- catabolism.
- Pathways that give rise to sugars, fatty acids, and amino acids all originate with components of the citric acid cycle.

Section 19.9

- The citric acid cycle is considered part of aerobic metabolism because of the link to the electron transport chain and oxidative phosphorylation.
- NADH and FADH2 generated by the citric acid cycle ultimately pass their electrons to oxygen.

LECTURE NOTES

The citric acid cycle plays a central role in both central energy metabolism and biosynthesis. As such, at least two lectures should be devoted to this chapter. The reactions all deserve careful treatment, including the entry of pyruvate into the cycle, as do the control mechanisms. Anaplerotic reactions and the glyoxylate cycle also show the amphibolic nature of the cycle.

LECTURE OUTLINE

I. Central role of the citric acid cycle
    A. Central energy metabolism – ETC and OxPhos
    B. Amphibolic nature – catabolism and anabolism
II. Overall Pathway
    A. Mitochondrial structure review
    B. Importance of oxidative decarboxylations
    C. Production of NADH and FADH2 for ETC
III. Pyruvate conversion to acetyl-CoA
    A. Pyruvate dehydrogenase complex
    B. Coenzyme A
    C. Pyruvate dehydrogenase
        1. Use of thiamine pyrophosphate
        2. Loss of CO2 leaving two-carbon unit
    D. Dihydrolipoyl transacetylase
        1. Amide linkage of lipoic acid to lysine
        2. Thioester linkage of acetyl to lipoic acid
        3. Transfer to CoA

E. Dihydrolipoyl dehydrogenase
    1. Reoxidation to disulfide form
    2. Use of FAD
    3. Transfer of electrons to NAD+
    4. 2 NADH/glucose = 5 ATP/glucose
F. Quaternary structure of complex

IV. The reactions of the citric acid cycle
A. Citrate formation
    1. Condensation reaction
    2. Oxaloacetate
    3. Citrate synthase
    4. Exergonic nature of reaction – release of CoA
B. Isomerization
    1. Aconitase
    2. Stereospecificity of product
    3. Requirement of Fe(II)
C. First oxidation
    1. Isocitrate dehydrogenase
    2. Production of NADH
    3. 2 NADH/glucose = 5 ATP/glucose
D. Second oxidation
    1. α-ketoglutarate dehydrogenase complex similarity to pyruvate dehydrogenase complex
    2. 2 NADH/glucose = 5 ATP/glucose
    3. Origin of CO2 molecules
E. Succinate formation
    1. Production of GTP
    2. Succinyl-CoA synthetase
    3. Transfer of phosphate between GTP and ATP
F. Fumarate formation
    1. Succinate dehydrogenase
    2. Integral membrane protein – inner mitochondrial membrane
    3. Use of FAD
    4. Non-heme iron protein
G. Malate formation
    1. Fumarase
    2. Stereospecific formation of L-isomer
H. Final oxidation
    1. Malate dehydrogenase
    2. Regeneration of oxaloacetate
    3. Production of NADH

I. Overall reactions
   1. Pyruvate dehydrogenase complex
   2. Citric acid cycle
   3. Overall from pyruvate to CO2
   4. Eventual ATP production

V. Energetics and control
   A. General thermodynamic considerations
   B. Control of pyruvate dehydrogenase
      1. Inhibition by ATP and NADH
      2. Activation by ADP
      3. Reversible phosphorylation
      4. Inhibition by acetyl-CoA
   C. Control of cycle proper
      1. Citrate synthase allosterically inhibited by ATP, NADH, succinyl-CoA, and citrate
      2. Isocitrate dehydrogenase allosterically activatd by ADP and NAD+
      3. α-ketoglutarate dehydrogenase complex inhibited by ATP, NAH, and succinyl-CoA
      4. "Energy charge" and overall control of metabolism

VI. Glyoxylate cycle
   A. Use of acetyl-CoA for carbohydrate synthesis in non-animals
   B. Isocitrate lyase and malate synthase
   C. Production of oxaloacetate from two molecules of acetyl-CoA
   D. Use of oxaloacetate in gluconeogenesis
   E. Glyoxysomes and importance in germinating seeds
   F. Importance in bacteria

VII. CAC and catabolism
   A. Stages of catabolism
   B. CAC as central point for catabolic pathways

VIII. CAC and anabolism
   A. Anaplerotic reactions – pyruvate carboxylase
   B. Lipid anabolism
   C. Anabolism of amino acids and other metabolites

IX. Link to oxygen

ANSWERS TO PROBLEMS

19.1 The Central Role of the Citric Acid Cycle in Metabolism

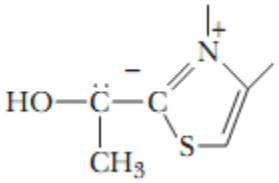
1. Anaerobic glycolysis is the principal pathway for the anaerobic metabolism of glucose. The pentose phosphate pathway can also be considered. Aerobic glycolysis and the citric acid cycle are responsible for the aerobic metabolism of glucose.
2. Anaerobically, two ATPs can be produced from one glucose molecule. Aerobically, this figure is 30 to 32, depending on in which tissue it is occurring.
3. The citric acid cycle is also called the Krebs cycle, the tricarboxylic acid cycle, and the TCA cycle.

4. Amphibolic means that the pathway is involved in both catabolism and anabolism.

19.2 The Overall Pathway of the Citric Acid Cycle

5. The citric acid cycle takes place in the mitochondrial matrix. Glycolysis takes place in the cytosol.
6. There is a transporter on the inner mitochondrial matrix that allows pyruvate from the cytosol to pass into the mitochondria.
7. NAD^+ and FAD are the primary electron acceptors of the citric acid cycle.
8. NADH and $FADH_2$ are indirect sources of energy produced in the TCA cycle. GTP is a direct source of energy.

19.3 How Pyruvate Is Converted to Acetyl-CoA

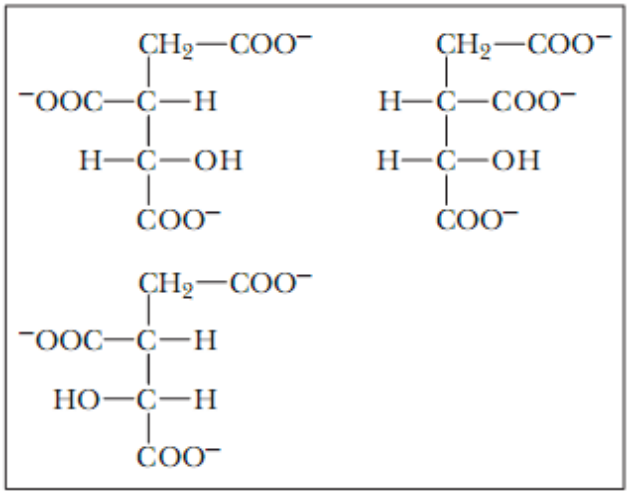
9. Five enzymes are involved in the pyruvate dehydrogenase complex of mammals. Pyruvate dehydrogenase transfers a two-carbon unit to TPP and releases CO_2. Dihydrolipoyl transacetylase transfers the two-carbon acetyl unit to lipoic acid and then to coenzyme A. Dihydrolipoyl dehydrogenase reoxidizes lipoic acid and reduces NAD^+ to NADH. Pyruvate dehydrogenase kinase phosphorylates PDH. PDH phosphatase removes the phosphate.
10. Lipoic acid plays a role both in redox and in acetyl-transfer reactions.
11. Five enzymes are all in close proximity for efficient shuttling of the acetyl unit between molecules and efficient control of the complex by phosphorylation.
12. Thiamine pyrophosphate comes from the B vitamin thiamine. Lipoic acid is a vitamin. NAD^+ comes from the B vitamin niacin. FAD comes from the B vitamin riboflavin.
13. 

14. See Figure 19.4.

19.4 The Individual Reactions of the Citric Acid Cycle

15. A condensation reaction is one in which a new carbon–carbon bond is formed. The reaction of acetyl-CoA and oxaloacetate to produce citrate involves formation of such a carbon–carbon bond.
16. It means that the reaction catalyzed by the enzyme produces the product that is part of the name and does not require a direct input of energy from a high-energy phosphate. Thus, citrate synthase catalyzes the synthesis of citrate without using ATP to do it.
17. Fluoroacetate is a poison that is produced naturally in some plants and is also used as a poison against undesirable pests. It is poisonous because it is used by citrate synthase to make fluorocitrate, which is an inhibitor of the citric acid cycle.
18. The reaction involves an achiral molecule (citrate) being converted to a chiral one (isocitrate).

19. Conversion of pyruvate to acetyl-CoA, conversion of isocitrate to α-ketoglutarate, and conversion of α-ketoglutarate to succinyl-CoA.
20. Conversion of pyruvate to acetyl-CoA, conversion of isocitrate to α-ketoglutarate, conversion of α-ketoglutarate to succinyl-CoA, conversion of succinate to fumarate, and conversion of malate to oxaloacetate.
21. These enzymes catalyze oxidative decarboxylations.
22. The reactions proceed by the same mechanism and use the same cofactors. The difference is the initial substrate, which is pyruvate or α-ketoglutarate. During the course of the reaction, pyruvate dehydrogenase shuttles an acetyl unit through the reaction while α-ketoglutarate dehydrogenase shuttles a succinyl unit.
23. A synthetase is an enzyme that synthesizes a molecule and uses a high-energy phosphate in the process.
24. In substrate-level phosphorylation, the energy of hydrolysis of some compound provides sufficient energy to allow the endergonic phosphorylation of ADP to ATP to take place. In the next chapter, we will see how the electron transport chain generates energy to allow the conversion of ADP to ATP.
25. We have previously encountered substrate-level phosphorylation in glycolysis. An example is the transfer of a phosphate from 1,3-*bis*phosphoglycerate to ADP to afford 3-phosphoglycerate and ATP
26. GTP is equivalent to ATP because an enzyme, nucleoside diphosphate kinase, is able to interconvert GTP and ATP.
27. The enzymes that reduce NAD^+ are all soluble, matrix enzymes, while succinate dehydrogenase is membrane-bound. The NAD^+-linked dehydrogenases all catalyze oxidations that involve carbons and oxygens, such as an alcohol group being oxidized to an aldehyde or aldehyde to carboxylic acid. The FAD-linked dehydrogenase oxidizes a carbon–carbon single bond to a double bond.
28. There is an adenine nucleotide portion in the structure of NADH, with a specific binding site on NADH-linked dehydrogenases for this portion of NADH.
29. The conversion of fumarate to malate is a hydration reaction, not a redox reaction.
30.



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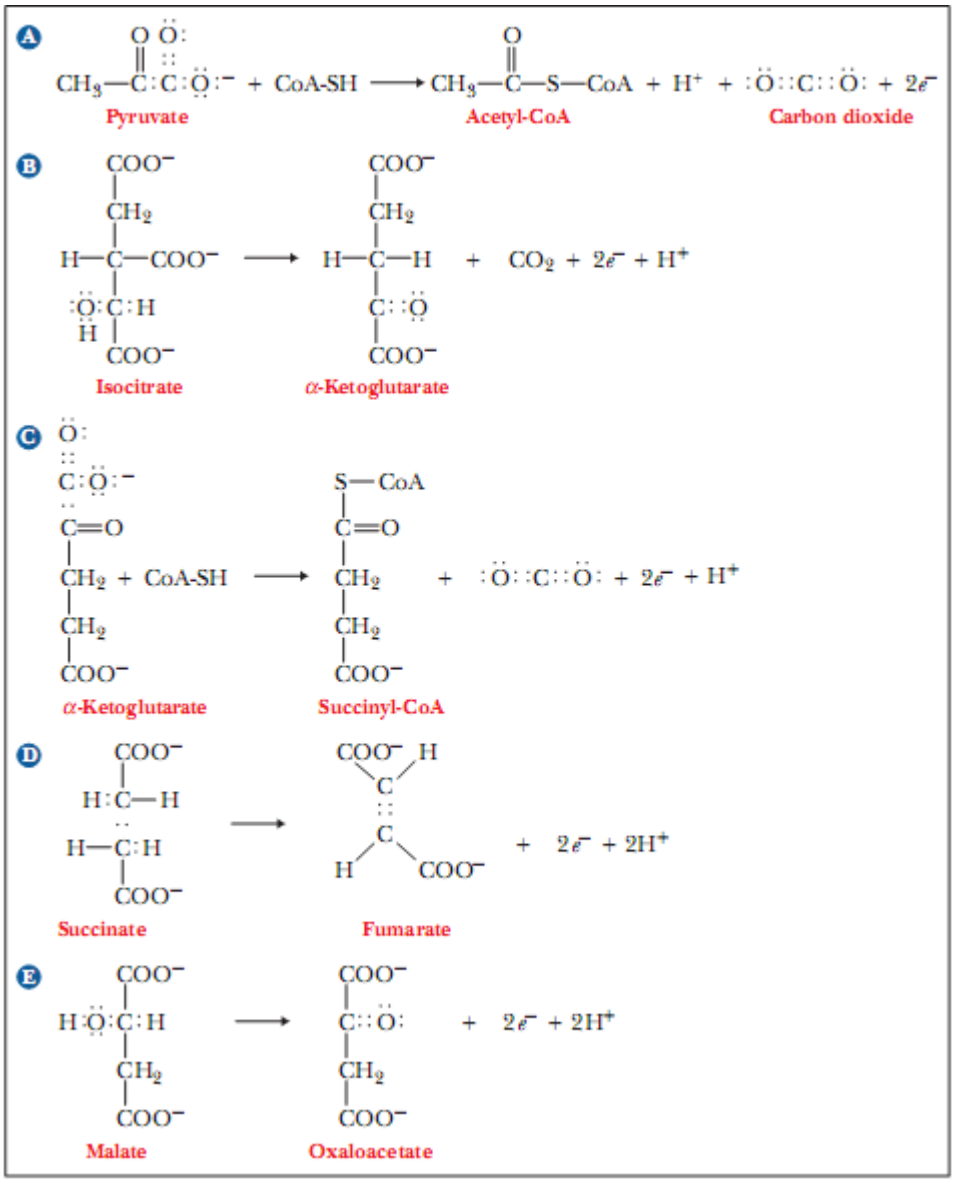

19.5 Energetics and Control of the Citric Acid Cycle

32. The reactions are catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.
33. PDH is controlled allosterically. It is inhibited by ATP, acetyl-CoA, and NADH. In addition, it is subject to control by phosphorylation. When PDH kinase phosphorylates PDH, it becomes inactive. Removing the phosphate with the PDH phosphatase reactivates it.
34. ATP and NADH are the two most common inhibitors.

35. If the amount of ADP in a cell increases relative to the amount of ATP, the cell needs energy (ATP). This situation not only favors the reactions of the citric acid cycle, which release energy, activating isocitrate dehydrogenase, but also stimulates the formation of NADH and $FADH_2$ for ATP production by electron transport and oxidative phosphorylation.
36. If the amount of NADH in a cell increases relative to the amount of NAD^+, the cell has completed a number of energy-releasing reactions. There is less need for the citric acid cycle to be active; as a result, the activity of pyruvate dehydrogenase is decreased.
37. The citric acid cycle is less active when a cell has a high ATP/ADP ratio and a high NADH/NAD^+ ratio. Both ratios indicate a high "energy charge" in the cell, indicating less of a need for the energy-releasing reactions of the citric acid cycle.
38. Thioesters are "high-energy" compounds that play a role in group-transfer reactions; consequently, their $\Delta G°'$ of hydrolysis is large and negative to provide energy for the reaction.
39. The energy released by hydrolysis of acetyl-CoA is needed for the condensation reaction that links the acetyl moiety to oxaloacetate, yielding citrate. The energy released by hydrolysis of succinyl-CoA drives the phosphorylation of GDP, yielding GTP.
40. Table 19.2 shows that the sum of the energies of the individual reactions is –44.3 kJ (–10.6 kcal) for each mole of acetyl-CoA that enters the cycle.
41. The expression would relate to the intensive extraction of energy from intermediate compounds by redox reactions. Including the pyruvate dehydrogenase reaction, 5 of 9 reactions are redox reactions (in contrast with only 1 of 10 in glycolysis). Accordingly, energy is rapidly extracted from carbon compounds (yielding the energyless CO_2) and is transferred to NAD^+ and FAD for subsequent utilization.
42. Lactose is a disaccharide of glucose and galactose. There is no energy cost in the hydrolysis of the bond between the two monosaccharides, so essentially there are two hexoses to consider. Because the processing of any of the hexoses yields the same amount of energy, the aerobic processing of lactose would lead to 60 to 64 ATPs, depending on the tissue and on the shuttle system used.

19.6 The Glyoxylate Cycle: A Related Pathway

43. Isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and succinyl-CoA synthetase.
44. The conversion of isocitrate to succinate and glyoxylate catalyzed by isocitrate lyase and the conversion of glyoxylate and acetyl-CoA to malate catalyzed by malate synthase.
45. Bacteria that have a glyoxylate cycle can convert the acetic acid to amino acids, carbohydrates, and lipids, but humans can use the acetic acid only as an energy source or to make lipids.

19.7 The Citric Acid Cycle in Catabolism

46. The citric acid cycle is the central metabolic pathway and indirect producer of energy. It receives fuels from the other pathways at many points and generates reduced electron carriers that go into the electron transport chain. It is also involved in anabolism, as many of its intermediates can be drawn off to synthesize other compounds.
47. The citric acid cycle occurs in the mitochondrial matrix, which is more selective in its permeability than the plasma membrane.
48. In oxidative decarboxylation, the molecule that is oxidized loses a carboxyl group as carbon dioxide. Examples of oxidative decarboxylation include the conversion of pyruvate to acetyl-CoA, isocitrate to α-ketoglutarate, and α-ketoglutarate to succinyl-CoA.
49. Yes, not only is citric acid completely degraded to carbon dioxide and water, but it is also readily absorbed into the mitochondrion.

19.8 The Citric Acid Cycle in Anabolism

50. The following series of reactions exchanges NADH for NADPH.
    Oxaloacetate + NADH + H^+ → Malate + NAD^+
    Malate + $NADP^+$ → Pyruvate + CO_2 + NADPH + H^+
51. A variety of reactions in which amino acids are converted to citric acid cycle intermediates are considered anaplerotic. In addition, pyruvate + CO_2 can form oxaloacetate via pyruvate carboxylase.
52. Many compounds can form acetyl-CoA, such as fats, carbohydrates, and many amino acids. Acetyl-CoA can also form fats and ketone bodies, as well as feed directly into the citric acid cycle.

19.9 The Link to Oxygen

53. The NADH and $FADH_2$ produced by the citric acid cycle are the electron donors in the electron transport chain linked to oxygen. Because of this connection, the citric acid cycle is considered part of aerobic metabolism.